INFORMATIONAL LEAFLET NO. 200

GENETIC VARIATION IN ALASKAN CHUM SALMON,

Oncorhynchus keta

By

Bob Davis

and

Carmen Olito

STATE OF ALASKA

Jay S. Hammond, Governor

DEPARTMENT OF FISH AND GAME

Ronald O. Skoog, Commissioner

P.O. Box 3-2000, Juneau 99802

April 1982

GENETIC VARIATION IN ALASKAN CHUM SALMON, *Oncorhynchus keta*

By

Bob Davis

and

Carmen Olito

Alaska Department of Fish and Game
Division of Fisheries Rehabilitation, Enhancement,
and Development
Anchorage, Alaska 99502

April 1982

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Eleven populations of chum salmon *(Oncorhynchus keta)* sampled in 1978 or 1979 were examined for activity of eighteen enzymes. Thirty-three genetic loci which provide the code for these enzymes were successfully identified in most of these populations. These data were collected to develop gene frequency profiles for use as quality control indicators in hatchery stocks, to investigate the genetic structuring of chum salmon populations, and to identify variant alleles which can be used as genetic markers in hatchery stocks or experimental treatment groups. Of the 33 loci examined, four (12%) were polymorphic with the variant allele frequency greater than .05. Two additional loci had rare alleles at less than .05 frequency.

Average heterozygosity ($\bar{H}$), was calculated for each population and genetic distance (D) for each pair of populations. Average heterozygosity for populations sampled in 1979 ranged from 3.0-3.5% with a mean of 3.2%. These values are similar to those reported in the literature for other populations and could serve as baseline data for quality control in these populations. Genetic distance ranged from .0002 to .0021 and was correlated with geographic distance ($r = .77$; $P < .01$).

Continued refinement of techniques and increase in number of populations and loci examined will provide a more accurate picture of the genetic structure of chum salmon stocks.

KEY WORDS: *Oncorhynchus keta*, population genetics, biochemical genetics, heterozygosity, genetic distance, genetic structure.

INTRODUCTION

A Fish Genetics Laboratory has been established within the Fisheries Rehabilitation, Enhancement, and Development Division, Alaska Department of Fish and Game to protect the genetic diversity of wild Pacific salmon *(Oncorhynchus* sp.) stocks and to increase the productive efficiency of hatcheries through the development and implementation of rational genetic policies and procedures. In order to accomplish this, a better understanding of the genetics of salmon, particularly the breeding structure of populations, is being sought through biochemical genetic screening of populations using the combined techniques of starch-gel electrophoresis and histochemical staining.

This enzyme methodology, developed by Hunter and Markert (1957) has since been used by population biologists to describe genetic variation present in natural populations. Utter and Hodgins (1970), Seeb and Grant (1976), Seeb and Wishard (1977), Grant (1977), Johnson (1979), and many other researchers have described biochemical genetic variation in North Pacific salmon populations. The applications of this technique to fisheries, particularly salmon management, have been reviewed by Utter et al. (1974) and Allendorf and Utter (1979).

This paper is a report of the work that has been done in Alaska on chum salmon *(O. keta)* populations in 1978 and 1979. Eleven chum salmon populations destined to become brood stock for State hatcheries were screened for genetic variation. The primary objective was to generate biochemical genetic profiles of these populations. From these profiles we can estimate genetic variation, genetic distance, identify populations or population groups to aid in stock separation, and provide insight into the possibility of genetic marking.

MATERIALS AND METHODS

The techniques of horizontal starch gel electrophoresis and histochemical staining have been outlined in detail by Utter et al. (1974) and May (1975). For better resolution under our conditions a few modifications on the described techniques were made.

Tissue Preparation

Eyes, heart, liver, and muscle samples were taken from freshly killed spawning chum salmon at 13 locations throughout Alaska (Tables 1 and 2). These samples were iced, frozen as soon as possible, and stored at -20°C until needed. A section of this frozen tissue was placed in a 12 x 75 mm glass tube with an equal amount of distilled water, ground with a glass rod, and refrozen. After thawing, the supernatant fluid was used for electrophoresis.

Electrophoresis

Twelve percent solutions of hydrolyzed potato starch and one of three buffer systems were used to make the gels: (1) A discontinuous tris, citric acid

Table 1. Alaskan chum salmon populations sampled in 1978.

Population	Location - Southeastern
Disappearance Creek	Prince of Wales Island East (Ketchikan)
Crystal Creek	Mitkof Island (South Petersburg)
W. Kupreanof Island[1] Tunehean Creek Irish Creek Hamilton River	Kupreanof Island (Petersburg)
Clear River	Baranof Island East (Sitka)
Kadashan Creek	Chichagof Island East
Prospect Creek	Mainland south of Juneau
Limestone Creek	Mainland south of Juneau

[1] 3 small populations pooled (N=18)

Table 2. Alaskan chum salmon populations sampled in 1979.

Population	Location
Crystal Creek	Southeast (Petersburg)
Crooked Creek	Prince William Sound (Valdez)
Kizhuyak River	Kodiak Island (Kodiak)
Russell Creek	Alaska Peninsula (Cold Bay)
Delta River	Interior - Fairbanks (Delta Junction)

gel (pH 8.1) and lithuim hydroxide, boric acid tray buffer (pH 8.0) described by Ridgway et al. (1970); (2) a citrate amine buffer (pH 6.1) described by Clayton and Tretiak (1972); and (3) a Tris, boric acid, EDTA buffer (pH 8.6) described by Markert and Faulhaber (1965). After the gels were set, filter paper wicks soaked in tissue extract were placed in a cut made 6 cm from one edge. Electric current was applied for 10 minutes, wicks removed, and electrophoresis resumed for approximately 4 hours.

Staining and Gel Interpretation

After electrophoresis was completed the starch gels were horizontally sliced into 1 mm sections with monofilament thread. Four slices from the center of the gel were placed in separate trays and stained for specific enzymes. The stain solutions were made according to Shaw and Prasad (1970) or Harris and Hopkinson (1977). The gel slice and stain were incubated in darkness at 45°C until bands became visible.

Results were interpreted within 24 hours and a photograph taken whenever possible to provide a permanent record. Variants were described, enumerated, and allelic frequencies calculated for each locus in each population. In describing the bands, the most common was designated 100 and the variants were given a value corresponding to their mobility relative to the common band.

Interpretation of Data

Electrophoresis sometimes produces variation in banding patterns that is not genetic. Therefore it is necessary to determine if observed variation should be interpreted as genetic or as an artifact of the technique. The most powerful evidence is the results of breeding experiments. If the progeny of parents with known biochemical differences display phenotypic ratios that conform to a single Mendelian genetic model, then the genetic basis is confirmed. Without breeding information several other lines of evidence can be used. Variation is assumed to be genetic if (1) the banding patterns resolved are interpretable on the basis of a simple genetic hypothesis; (2) variation is repeatable in the same tissue of an individual; and (3) when the locus is expressed in more than one tissue, the pattern is consistent among tissues.

Statistical Procedures

Gene frequencies were calculated after the different genotypes were enumerated. Deviations in genotypic frequencies from the Hardy-Weinburg equilibrium were detected using Chi-square test for goodness of fit.

Average heterozygosity, an estimate of the amount of genetic variation present, was calculated by 1-J(x) where J(x) is the arithmetic mean of j(x), and $j(x) = \Sigma x_i^2$ where x_i is the frequency of the ith allele in population X.

Genetic distance between populations was calculated using Nei's method (1972). Genetic distance (D) is defined as

$$D = -\log_e I \quad \text{and} \quad I = \frac{J(xy)}{\sqrt{J(x)J(y)}}$$

J(xy), J(x), and J(y) are the arithmetic mean of j(xy), j(x), and j(y) where $j(xy) = \Sigma x_i y_i$, $j(x) = \Sigma x_i^2$, and $j(y) = \Sigma y_i^2$ respectively. x_i and y_i are the allelic frequencies of populations X and Y are the ith locus. I is the genetic identity between populations X and Y.

To determine if the genetic distances between populations were significantly different from zero, a chi-square test developed by Nei and Roychoudhury (1974) was used. The x^2 values were calculated by:

$$x^2 = N_x N_y \Sigma[(x_i - y_i)^2 (x_i N_x + y_i N_y)^{-1}]$$

for each locus and summed over all loci examined. The degrees of freedom are equal to the number of alleles minus one.

RESULTS

Eighteen enzymes encoded by thirty-three loci were successfully identified in most of the eleven populations examined. These enzymes, their abbreviations, and the number of loci coding them are listed in Table 3. For comparisons between populations only twenty of these loci could be used. If no activity could be detected at one of these loci in a population, the locus was assumed to be monomorphic to minimize any differences. Of the 33 loci examined, four (12%) were polymorphic with the variant frequency greater than .05. An additional two loci had a rare variant at a less than .05 frequency. The frequencies of the alleles at each polymorphic locus are given in Table 4.

6-phosphogluconate dehydrogenase (6PGDH). This enzyme exhibited the three-banded heterozygous and the single-banded common homozygous patterns characteristic of a dimeric enzyme. Because the alternate allele frequency was low we did not see any of the alternate homozygous pattern. In five of the populations the incidence of the variant allele was less than 5%. Crooked Creek, with a small sample size, had a variant frequency of 10%.

Phosphomannose isomerase (PMI). This locus was resolved only in populations screened in 1979. All three banding patterns characteristic of a monomer were found in all populations. Assuming the banding patterns represent genotypes, frequencies in all populations fit those expected under Hardy-Weinburg equilibrium. The variant allele was often faint and had the same mobility as the common 6PGDH allele which sometimes appeared on PMI stained gels. Breeding studies are necessary here to verify that this variation is genetic and not an artifact of the techniques used.

Peptidase B (PEP-lgg, leucylglycylglycine). This locus had the three phenotypes characteristic of a monomeric enzyme in all populations screened. This enzyme could not be resolved clearly enough to be interpreted in three of the populations, although there was obviously some variation present. The variant allele frequency ranged between .04 and .17 but no obvious geographic trends could be established.

Table 3. Genetic loci resolved.

Enzyme	Number loci
Adenosine deaminase (ADA)	1
Adenylate kinase (AK)	1
Alcohol dehydrogenase (ADH)	1
α-glycerophosphate dehydrogenase (AGP)	3
Creatine kinase (CK)	3
Diaphorase (DIA)	1
Esterases (EST)	2
Isocitrate dehydrogenase (IDH)	1
Lactate dehydrogenase (LDH)	5
Leucine amino peptidase (LAP)	1
Peptidases (PEP)	3
6-phosphogluconate dehydrogenase (6PGDH)	1
Phosphoglucose isomerase (PGI)	3
Phosphoglucomutase (PGM)	1
Phosphomannose isomerase (PMI)	1
Pyruvate kinase (PK)	1
Sorbitol dehydrogenase (SDH)	2
Superoxide dismutase (SOD)	2
Total number of loci	33

Table 4. Chum salmon gene frequencies, standard error, and population size for each polymorphic locus.

LOCUS		POPULATION											
		Disappear.		Crystal		Kupreanof		Clear		Kadashan		Prospect	
AGP3	N	50		60		18		66		21		27	
	100	1.0		1.0		1.0		1.0		1.0		1.0	
	111	-	-	-	-	-	-	-	-	-	-	-	-
IDH	N	55		54		17		72		53		26	
	A	.46	.05	.38	.05	.38	.08	.42	.04	.45	.05	.54	.07
	B	.34	.05	.59	.05	.35	.08	.51	.04	.42	.05	.35	.07
	C	.10	.03	0	-	.06	.04	.02	.01	.03	.02	.02	.02
	D	.10	.03	.03	.02	.21	.07	.05	.02	.10	.03	.10	.04
LDH4	N	50		60		18		78		63		27	
	100	.99	.01	1.0	-	.97	.03	1.0	-	.99	.01	1.0	-
	137	.01	.01	0	-	.03	.03	-	-	.01	.01	-	-
PEP (B)	N	-		60		18		66		64			
	100	-	-	.87	.03	.92	.05	.96	.02	.88	.03	-	-
	71	-	-	.13	.03	.08	.05	.04	.02	.12	.03	-	-
6PGDH	N	50		50		14		75		63		26	
	100	.99	.01	.98	.01	1.0	-	.98	.01	1.0	0	1.0	0
	85	.01	.01	.02	.01	0	-	.02	.01	-	-	-	-
PMI	N			60									
	100	-	-	.79	.04	-	-	-	-	-	-	-	-
	85	-	-	.21	.04	-	-	-	-	-	-	-	-

-Continued-

Table 4. Chum salmon gene frequencies, standard error, and population size for each polymorphic locus (continued).

LOCUS		POPULATION									
		Limestone		Crooked		Kishuyak		Russell		Delta	
AGP3	N	68		20		28		89		49	
	100	.99	.01	1.0	-	1.0	-	1.0	-	1.0	-
	111	.01	.01	-	-	-	-	-	-	-	-
IDH	N	46		16		25		76		48	
	A	.48	.05	.53	.09	.54	.07	.52	.05	.57	.05
	B	.42	.05	.44	.09	.40	.07	.38	.04	.39	.05
	C	.06	.02	-	-	.04	.03	.05	.02	.04	.02
	D	.04	.02	.03	.03	.02	.02	.05	.02	-	-
LDH4	N	67		20		28		89		49	
	100	1.0	-	1.0	-	1.0	-	1.0	-	1.0	-
	137	-	-	-	-	-	-	-	-	-	-
PEP (B)	N			20		26		89		49	
	100	-	-	.93	.04	.87	.05	.85	.03	.83	.04
	71	-	-	.07	.04	.13	.05	.15	.03	.17	.04
6PGDH	N	51		20		26		88		49	
	100	.99	.01	.90	.05	1.0	0	1.0	0	.95	.02
	85	.01	.01	.10	.05	-	-	-	-	.05	.02
PMI	N	61		17		26		88		49	
	100	1.0	-	.79	.07	.83	.05	.92	.02	.94	.02
	85	-	-	.21	.07	.17	.05	.08	.02	.06	.02

α-glycerophosphate dehydrogenase (AGP-3). Three loci provide the code for this enzyme in chum salmon (Utter, personal communication). Limestone Creek in Southeastern Alaska was the only population with any variability, and this was restricted to one heterozygote, showing the typical dimeric banding pattern at the third locus.

Lactate dehydrogenase (LDH). Five LDH loci are recognized in salmonids (Morrison and Wright, 1966; Utter, Allendorf, and Hodgins 1973). LDH-4, best expressed in liver, has a fat variant allele at a frequency less than .05. Only the common and heterozygous genotypes were seen in Southeastern Alaska while other populations were all monomorphic. This is consistent with the findings of Utter, Allendorf, and Hodgins (1973) in North Pacific and Washington State chum salmon.

Isocitrate dehydrogenase (IDH). There are two IDH loci in chum salmon. IDH-2, a dimer best expressed in liver, is polymorphic for four alleles. The four allele model used to describe this system is one explained by Utter (personal communication). Two alleles, A and B, are most common in all populations with frequencies near .5. The frequency of the B allele in most Southeastern populations exceeded .5. This is obviously different from the other populations examined where the A allele predominates. The frequency of the D allele may be underestimated here as its activity was slightly diminished, hence much more difficult to detect.

Average heterozygosity ($\underline{H}$) was estimated for each chum salmon population examined (Table 5). Average heterozygosity, which represents the average proportion of heterozygotes per locus, is a direct estimate of genetic variation in a population (Selander and Johnson 1973; Allendorf and Utter 1979). In 1978 populations $\underline{H}$ ranged from .0174 to .0270 (Mean = .0264) and in 1979 populations $\underline{H}$ ranged from .0298 to .0354 (Mean = .0321).

Genetic distance was calculated for all combinations of the eleven populations examined (Table 5). Populations collected in Southeastern Alaska (Crystal Lake collected in 1979, all others in 1978) ranged in genetic distance from .0002 to .0033. When the Crystal Lake sample is eliminated from the Southeastern samples collected in 1978, genetic distance ranges from .0002 to .0009. The populations collected in 1979 cover a much wider geographic area and genetic distance ranges from .0002 to .0020.

DISCUSSION

Gene frequency profiles of biochemical genetic loci can be used for population and stock separation studies; variant alleles can also be used as genetic markers in hatchery stocks or experimental treatment groups. Our primary reason for collecting frequency profiles is for use as quality control indicators in the Alaska Department of Fish and Game hatchery programs.

The concensus among geneticists is that heterozygosity enhances fitness (viability, vigor, fecundity, fertility, etc.). This belief is based on both the results of laboratory experiments and the experience of plant and animal

Table 5. Genetic distance, genetic identity, and average heterozygosity of chum salmon populations sampled in 1978 and 1979.

Nei's genetic distance above diagonal
Average heterozygosity on diagonal in ( )
Genetic identity below diagonal

	1	2	3	4	5	6	7	8	9	10	11
1. Disappearance	(.0210)	.0032*	.0005	.0007	.0006	.0002	.0002	.0022*	.0017*	.0011*	.0015*
2. Crystal	.9968	(.0334)	.0003*	.0017*	.0020*	.0033*	.0025*	.0010	.0011	.0016*	.0020*
3. Kupreanof	.9995	.9970	(.0270)	.0009	.0004	.0008	.0009	.0028	.0020	.0011	.0018*
4. Clear	.9993	.9983	.9991	(.0205)	.0004	.0007	.0003	.0019*	.0016*	.0010*	.0013*
5. Kadashan	.9994	.9980	.9996	.9996	(.0255)	.0006	.0005	.0020*	.0011*	.0004	.0007
6. Prospect	.9998	.9967	.9992	.9993	.9994	(.0174)	.0002	.0020	.0015	.0010	.0013*
7. Limestone	.9998	.9975	.9991	.9997	.9995	.9998	(.0190)	.0019*	.0015*	.0009*	.0012*
8. Crooked	.9978	.9990	.9972	.9981	.9980	.9980	.9982	(.0354)	.0005	.0011*	.0012
9. Kizhuyak	.9982	.9989	.9980	.9984	.9989	.9985	.9985	.9995	(.0320)	.0003	.0005
10. Russell	.9989	.9984	.9989	.9990	.9996	.9991	.9991	.9989	.9997	(.0298)	.0002
11. Delta	.9985	.9980	.9982	.9987	.9993	.9987	.9988	.9988	.9995	.9998	(.0306)

*Genetic distance significantly different from 0.

breeders. A decrease in genetic variation is therefore expected to result in a reduction of fitness. Average heterozygosity, which we have calculated for all populations, is a direct measure of genetic variation. Mutation, migration (gene flow), selection, inbreeding, and small population size or population bottlenecks are all forces that may change the genetic structure of a population. In wild populations these forces are apparently in equilibrium, and the genetic structure is usually stable. Allendorf and Utter (1979) reported that average heterozygosities calculated for a variety of hatchery and wild rainbow trout populations were, with but one exception, consistent. The one exception was a hatchery population with an extremely low average heterozygosity. Artificial selection, inbreeding, and small population numbers are most likely to cause loss of genetic variation in a hatchery stock. The exceptional population is known to have been subjected to intense artificial selection, and as a consequenc-, reduced population size. With proper management of hatchery brood stocks this statistic ($\underline{H}$) should remain constant.

Over the 2 years in which genetic data were collected, average heterozygosities were not constant. In 1979 populations, $\underline{H}$ ranged from 3.0-3.5%, remaining relatively consistent. The mean taken over all stocks for 1979 data was $\underline{H}$ = 3.2%. This is identical to that reported by Altukov in work done in the Soviet Union (reported in Allendorf and Utter 1979). The inconsistencies in the 1978 populations probably reflect either an inadequate sample size or, more likely, an initial lack of familiarity with the techniques used. With continual refinement of the techniques and an increase in the number of loci examined, a better estimate of $\underline{H}$ will be attained, hence a more accurate picture of the biochemical genetic structure of chum salmon brood stocks.

In addition to use as quality control indicators, genetic profiles provide valuable information on the genetic structure of wild populations. Genetic distances were calculated for all population combinations (Table 5, 6, and 7). A genetic distance of D = 0 occurs when two populations have the same alleles with the same frequencies at all loci; and D = 1 indicates that they have no common alleles at any locus. If the two stocks being compared are sexually isolated (i.e., straying = 0), and the rate of gene substitution per locus is the same for all loci, D would measure the total number of gene substitutions or codon differences per locus (Nei 1972). Apparently neither of the assumptions is true. However, genetic distance is a relative measure of genetic divergence or population structuring.

Crystal Creek in Southeastern Alaska was sampled in both 1978 and 1979. Because the data for 1979 were much more complete and, therefore, more reliable, 1979 Crystal Lake gene frequency data were used in calculating all comparisons. The significant differences between Crystal Creek and Southeastern Alaska chum populations collected in 1978 are probably a reflection of sample and technique differences rather than true genetic distances. Table 5 was broken down by year sampled, and each year's data were examined separately (Tables 6 and 7). Samples collected in 1978 were all from Southeastern Alaska stocks. This may suggest that Southeastern chum salmon are homogeneous, but it probably reflects poor samples, problems with the technique in 1978 data as suggested above, or both. The low average heterozygosity estimates seem to indicate that problems of technique were the primary cause.

Table 6. Genetic distance and average heterozygosity of chum salmon populations sampled in 1978.

Nei's "D" (Genetic Distance) above the diagonal.
H (Average Heterozygosity) on the diagonal.

	(1)	(3)	(4)	(5)	(6)	(7)
Disappearance (1)	(.0210)	.0005	.0007	.0006	.0002	.0002
W. Kupreanof (3)		(.0270)	.0009	.0004	.0008	.0009
Clear (4)			(.0205)	.0004	.0007	.0003
Kadashan (5)				(.0255)	.0006	.0005
Prospect (6)					(.0174)	.0002
Limestone (7)						(.0190)

Table 7. Genetic distance and average heterozygosity of chum salmon populations sampled in 1979.

Nei's "D" (Genetic Distance) above the diagonal.
H (Average Heterozygosity) on the diagonal.

	(2)	(8)	(9)	(10)	(11)
Crystal (2)	.0334	.0010	.0011	.0016*	.0021*
Crooked (8)		.0354	.0005	.0011*	.0012
Kizuyak (9)			.0320	.0003	.0006
Russell (10)				.0298	.0002
Delta (11)					.0307

*Genetic distance significantly different from 0.

When comparing the populations collected, three were significantly different from zero (Table 7). These indicate that there are real differences between populations at least when samples are taken over a broad geographic area. When all 1979 distance values are included there is a correlation between genetic distance and geographic distance ($r = .77$). This relationship can be adequately explained by random genetic drift without invoking natural selection or environmental clines (Christiansen and Frydenberg 1974). [Recall that genetic drift refers to chance variation in allele frequency. These fluctuations are most likely to occur as a result of random sampling among gametes in small populations (sampling error)]. Sampling additional populations and more complete frequency data may show other geographic patterns and systematic relationships among populations.

REFERENCES

Allendorf, F.W. and F.M. Utter. 1979. Population Genetics. In: Fish Physiology, Vol. VIII: 407-454. Academic Press, N.Y.

Christiansen, F.B. and O. Frydenberg. 1974. Geographical patterns of four polymorphisms in Zoarces viviparus as evidence of selection. Genetics 77: 765-770.

Clayton, J.W. and D.N. Tretiak. 1972. Aminecitrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Bd. Canada. 29: 1169-1172.

Grant, S. 1977. Biochemical genetic variation in sockeye salmon: the use of biochemical markers for stock identification in Bristol Bay and Cook Inlet, Alaska. Final Report to Alaska Dept. Fish and Game, Comm. Fish. Service contract no. 3131. 38 pp.

Harris, H. and D.A. Hopkinson. 1977. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland Publishing Company, Amsterdam, Netherlands.

Hunter, R.L. and C.L. Markert. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science 125: 1294-1295.

Johnson, K.R. 1979. Genetic variation in populations of pink salmon *(Oncorhynchus gorbuscha)* from Kodiak Island, Alaska. M.S. Thesis, University of Washington.

Markert, C.L. and I. Faulhaber. 1965. Lactate dehydrogenase enzyme patterns of fish. J. Exp. Zool. 159: 319-332.

May, B. 1975. Electrophoretic variation in the genus *Oncorhynchus*: the methodology, genetic basis, and practical applications to fisheries research and management. M.S. Thesis. University of Washington.

Morrison, W.J. and J.E. Wright. 1966. Genetic analysis of three lactate dehydrogenase isozyme systems on trout: evidence for linkage of genes coding subunits A and B. J. Exp. Zool. 163(3): 259-270.

Nei, M. 1972. Genetic distance between populations. Amer. Nat. 106: 283-292.

_____ and A.K. Roychoudhury. 1974. Sampling variance of heterozygosity and genetic distance. Genetics 76: 379-390.

Ridgway, G.J., S.W. Sherburne, and R.D. Lewis. 1970. Polymorphism in the esterases of Atlantic herring. Trans. Amer. Fish. Soc. 99: 147-151.

Seeb, J. and S. Grant. 1976. Biochemical genetic variation in coho, chinook, chum, and pink salmon: the use of electrophoretic markers in stock identification. Final Report, Service Contract No. 711. Nat. Mar. Fish. Service, Seattle, Washington.

Seeb, J., and L. Wishard. 1977. Genetic characterization of Prince William Sound pink salmon populations. Report to Alaska Dept. Fish and Game. 21 pp.

Selander, R.K. and W.E. Johnson. 1973. Genetic variation among vertebrate species. Ann. Rev. Ecology and Syst. 4:75-91.

Shaw, C.R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes: a compilation of recipes. Biochem. Genet. 4:297-320.

Utter, F.M. and H.O. Hodgins. 1970. Phosphoglucomutase polymorphism in sockeye salmon. Comp. Biochem. Physio. 36: 195-199.

_____, F.W. Allendorf, and H.O. Hodgins. 1973. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. Syst. Biol. 22: 257-270.

_____, H.O. Hodgins, and F.W. Allendorf. 1974. Biochemical genetic studies of fishes: Potentialities and limitations. p. 213-238. In: D.C. Malins and J.R. Sargent (eds.). Biochemical and Biophysical Perspectives in Marine Biology. Academic Press, New York, N.Y.